PHAGE THERAPY

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Bacteriophages - viruses that kill bacteria - were first identified in the early part of the 20th century by Frederick Twort and Felix d'Herelle who called them *bacteriophages* or bacteria-eaters (from the Greek *phago* meaning *to eat* or *to devour*). Bacteriophages (or 'phages' for short) are ubiquitous, obligate parasites highly specific for their bacterial host. Because of their remarkable antibacterial activity, phages were used to treat diseases of humans and agriculturally-important animals almost immediately after their discovery. The first reported application of phages to treat infectious diseases of humans was by Bruynoghe and Maisin in 1921, who successfully used bacteriophages to treat staphylococcal skin disease. In the 1930-1940s, *Eli Lily and Co*. manufactured several therapeutic phage products. Other major companies involved in therapeutic phage production included *E.R. Squibb and Sons* and *Swan-Myers* (Abbot Laboratories). However, with the advent of antibiotics, the initially strong interest in phage therapy declined in the West. Overseas activity survived longer. The Russian and German armies routinely used phage preparations, and from the 1920s to the current day, phage therapy has been utilized in Eastern Europe and the former Soviet Union.

Phages have been used against cholera in the historic "Cholera Study" in India. *Shigella* phages have been correlated with the decreased incidence of dysentery. Numerous additional publications – most of them published in non-English scientific literature – report on various applications of bacteriophages in clinical settings. Some of the commercial phage products from the Laboratoire du Bactériophage in France included *Bacté-coli-phage*, *Bacté-rhino-phage*, *Bacté-intesti-phage*, *Bacté-pyo-phage*, and *Bacté-staphy-phage*, Eli Lily has *Colo-lysate*, *Ento-lysate*, *Neiso-lysate*, and *Staphylo-lysate*, *Colo-jel*, *Ento-jel*, and *Staphylo-jel*. Currently, ImBio in Russia is producing phage-based preparations in liquid, tablet, and cream formulations for treating bacterial dysentery (**"**Bacteriophagum dysentericum polyvalentum in tabulettis"), the early stages of salmonellosis ("Bacteriophagum salmonellae gr.ABCDE liquidum et siccum cum indumento acidoresistentis"), general gastrointestinal disorders ("Bacteriophagum coliproteicum liquidum"), *S. aureus* infections ("Bacteriophagum staphylococcus**"**), and *P. aeruginosa* infections ("Bacteriophagum *Pseudomonas aeruginosa* liquidum"). Another company in Russia, Biophag, currently manufactures at least two complex phage preparations ("Bacteriophagum" and "Piobacteriaphagum") targeting various bacterial pathogens. CMBP in Georgia produces *PhagoBioDerm*, and Eliava Institute of Bacteriophage sells several therapeutic phage preparations, including *IntestiPhage* and *PyoPhage*.

From a clinical standpoint, phages are very safe. This is not surprising, given that humans are exposed to phages from birth (and, possibly, even *in utero*). Indeed, bacteriophages are arguably the most ubiquitous organisms on earth. In the USA, approximately 3×10^9 coliphages are shed per person per day, which extrapolates to approximately 780,000,000,000,000,000 coliphages shed daily in this country alone. One ml of non-polluted water contains 2×10^8 PFU of phages, and the total number of phages

on earth is estimated to be $1x10^{30} – 1x10^{32}$. Phages are abundant in saltwater, freshwater, soil, plants and animals, and they even have been isolated from some vaccines and sera commercially available in the United States. Also, phages are commonly isolated from foods consumed by humans and other animals, they are normal commensals of the human body, and they have been commonly found in the human gastrointestinal tract, skin, urine, and mouth, where they are harbored in saliva and dental plaque. The abundance of phages in the environment – and the continuous exposure of humans to them – explains the extremely good tolerance of the human organism to phages. Indeed, during the approximately 80 years of therapeutic phage applications, phages have been administered to humans orally, in tablet or liquid formulations (10^5 - 10^{11} PFU/dose), rectally, locally (skin, eye, ear, nasal mucosa, etc.), in tampons, rinses and creams, as aerosols or intrapleural injections, and intravenously – and there have been no reports of serious complications associated with their use. Because of this apparent safety of phages, phage phi X174 has been used in the United States to monitor immune function in patients, including immunocompromised patients.

Phage preparations are currently being developed in the United States for use in animal husbandry and other agricultural settings, to control enteric pathogens and improve food safety. For example, phage cocktail PLSV-1 significantly reduces the incidence of *Salmonella* in broiler ceca, and phage LMP-102 significantly reduces levels of *L. monocytogenes* on various foods. In the human therapy area, the PhagoBioDerm preparation has been in trials in Georgia (one of the former Soviet Union republics) recently, showing reduction of MRSA in humans - a trial that exemplifies the potential value of phages to address untreatable or difficult-to-treat antibiotic resistant infections.

The mode of action of phages is due to specific and effective lysing of targeted bacteria; secondary mechanism of action may involve immune stimulation through the components of phage lysate. In this context, phages and phage-encoded enzymes are also utilized to kill preparations of bacteria for use as killed vaccines or to develop so called "bacterial ghost" vaccines. Immediate research is required to determine the value of presently used phage therapy in various pre-clinical and clinical settings (which, technologically, could be fairly rapidly adapted for clinical applications in the West). Also, phage-encoded lytic enzymes could be used as antibacterial agents (which may require a longer development period). Furthermore, phages and their encoded EPS-degrading enzymes may be active against bacterial biofilms and bacteria embedded in biofilms (e.g., *P. aeruginosa* and CF). Finally, lytic mechanisms of phages could be used to identify novel drug targets (which is the most long-term, but still very intriguing, approach).

The standard panel of controlled efficacy studies, pharmacokinetics, and compatibility with other interventions such as antibiotics, probiotics, etc. should be ascertained. The impact (short-term and long-term) on the normal microflora and interactions with bacterial biofilms remains unknown presently. The study of phage genomes and elucidating the role of various phage genes in the lytic cycle, and the study of the Phage-Bacteria interaction mechanisms and the pathways involved in the lytic cycle**,** may identify novel therapeutic targets or novel class of therapeutic agents.